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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Distinct characteristics of COVID-19 patients with initial rRT-PCR-positive and rRT-PCR-negative results for SARS-CoV-2

To the Editor,

Since the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) first emerged in Wuhan, China, in December 2019, the outbreak of COVID-19 epidemic has become an increasingly serious global health concern. Currently, over 150 countries have reported COVID-19 cases, and the situation has progressed to a pandemic associated with substantial morbidity and mortality.[1]

At present, rRT-PCR (real-time reverse transcription–polymerase chain reaction) assay is the most common and only direct method of SARS-CoV-2 detection for the diagnosis of COVID-19.[2] However, false-negative results due to laboratory errors or improper collection of the specimens may inevitably lead to an important percentage of undiagnosed COVID-19 patients. Some patients with suspected COVID-19 have initial negative result of the rRT-PCR test[3,4] or low viral concentration of SARS-CoV-2 at the sampling site in the early stages of the disease.[5] This study aims to compare the clinical and laboratory characteristics of eventually confirmed COVID-19 patients with initial positive and negative SARS-CoV-2 nucleic acid test results.

The present study retrospectively reports 290 laboratory-confirmed COVID-19 patients hospitalized from December 29, 2019, to February 16, 2020. According to initial rRT-PCR results, patients were divided into initial positive and negative groups. All rRT-PCR assays were performed with the same kit (Shanghai bio-germ Medical Technology Co Ltd). Electronic medical records, including patients' demographics, clinical manifestation, comorbidities, laboratory results, and radiological materials on admission were collected and analyzed. The clinical outcomes of each patient were reviewed and analyzed on the final follow-up (February 28, 2020) including disease severity, complications, and co-infection status with other pathogens during hospitalization. This study was approved by the Zhongnan Hospital of Wuhan University institutional ethics board (No.2020015 and No.2020028). Additional details on the methods are reported in the Supporting Information.
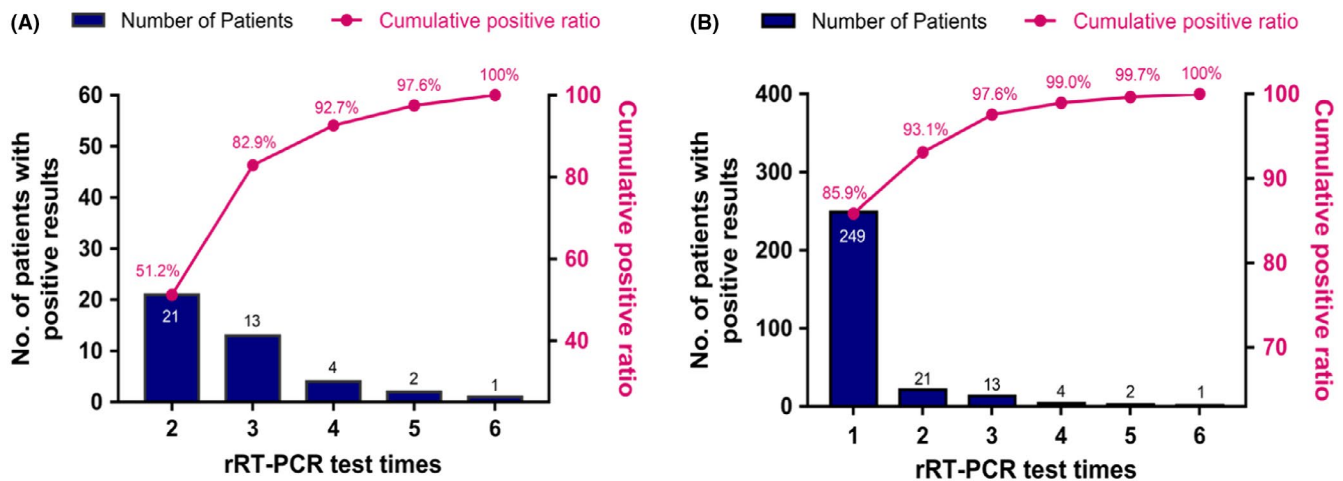
FIGURE 1 Number of patients testing positive for SARS-CoV-2 and the cumulative positive ratio at different rRT-PCR tests. A, Changes in the outcome of the rRT-PCR test of patients, from negative to positive, after consecutive assays. Of the 41 patients with an initial negative result, 21, 13, 4, 2, and 1 patients were tested positive at the 2nd, 3rd, 4th, 5th, and 6th rRT-PCR assays, respectively. The cumulative positive rRT-PCR assay ratio from the second to the fifth test increased from 51.2% (21/41) to 97.6% (40/41). B, Number and percentage of positive results within the total patient population. The cumulative positive ratio of all 290 patients from the first to the fifth test increased from 85.9% (249/290) to 99.7% (289/290). rRT-PCR, real-time reverse transcription–polymerase chain reaction

Of the 290 patients involved in this study, an initial rRT-PCR test was positive for SARS-CoV-2 by 249 (85.9%) patients and negative by 41 (14.1%) patients. Among the 41 patients with an initial negative result, 21 tested positive at the second rRT-PCR test and 13 of them became positive at the third test. The cumulative positive ratio of rRT-PCR assay of these 41 patients from the 2nd time to the 5th time of the test gradually increased from 51.2% (21/41) to 82.9% (34/41), 92.7% (38/41), and 97.6% (40/41) (Figure 1A), and the rRT-PCR-positive ratio of all 290 patients by the 5th test was 99.7% (289/290) (Figure 1B).

The median age of the patients was 57 years (range, 22-88 years), and 184 (63.5%) were aged over 50 years (Table 1). Of the 290 patients, 155 (53.4%) patients were male, but the majority [25 (61%)] of the patients with initial negative rRT-PCR results were female. Of the 89 (30.7%) patients with surgery history, the proportion of patients with an initial positive rRT-PCR result was significantly higher than that of patients with an initial negative rRT-PCR result (32.9% vs 17.1%, $P = .041$).

Compared to patients with an initial negative rRT-PCR result, patients with an initial positive result were more likely to progress to a severe condition (44.6% vs 24.4%, $P = .015$) (Table 1). In 226 patients with available chest CT images, 214 (94.7%) had typical patterns of COVID-19, and the proportions for initially positive and negative patients with abnormal CT images were 94.2% (180/191) and 97.1% (34/35), respectively. On February 28th, out of the 290 patients, 189 (65.2%) were discharged and, unfortunately, 46 (15.9%) did not survive. The mortality of patients with an initial negative rRT-PCR result [5 (12.2%)] was slightly lower than those with a positive result [41 (16.5%)], whereas the proportion of discharged patients from the negative group [32 (78%)] tended to be larger than the positive group [157 (63.1%)], without any statistically significant difference (Table S1).

The laboratory results of patients on admission are shown in Table S2. Among 290 patients, leukopenia (n = 78, 26.9%), neutropenia (n = 51, 17.6%), lymphopenia (n = 203, 70.0%), eosinopenia (n = 171, 59.0%), and thrombocytopenia (n = 65, 22.4%) were observed, without any significant difference between patients with initial positive and negative rRT-PCR results. Elevated levels of C-reactive protein (n = 249, 88%), serum amyloid A (n = 149, 84.2%), procalcitonin (n = 85, 31.1%), D-dimer (n = 105, 45.3%), and serum creatine kinase (n = 30, 15.7%) were found. No significant difference was seen between the initial positive and negative patients within these acute phase reactants (all $P > .05$) (Table S2).

In general, except for two aspects (surgery history and severity), no significant difference was observed in clinical manifestations, laboratory findings, and radiological changes between patients with initial positive and negative rRT-PCR results in the present study. Similar results were reported by Li et al and Lu et al,[6,7] where patients with a positive rRT-PCR test appeared to have increased disease severity, although the differences were not statistically significant in Li's (35.5% vs 13%, $P = .063$) and Lu's (20.8% vs 13.7%, $P = .20$) study. Surgery history may be a confounding factor in this study, which may also relate to factors such as age and sex. The findings of further logistic regression analysis showed no predictive value for the history of surgery.

Previous studies suggested that a false-negative rRT-PCR result may occur in some COVID-19 patients.[3,4] False-negative results may occur as a result of various factors, such as human errors when following the diagnostic kit protocol, the sensitivity of reagents, the site and method of specimen sampling and collection times.[8] It should be noted that a fraction of initial rRT-PCR negative results may be due to low or no virus expression in the sampled area. In regard to the site of specimen sampling, it has been previously reported that the viral load in the nose is higher than in the pharynx, and the virus detection rate in the lower respiratory

TABLE 1 Demographics and partial clinical data of patients with COVID-19

	All Patients (n = 290)	Initial result of SARS-CoV-2 rRT-PCR test		
		Positive (n = 249)	Negative (n = 41)	P value
Age—median (range)	57 (22-88)	57 (22-88)	51 (22-79)	.089
Age groups -No. (%)				
<30 y	16 (5.5)	11 (4.4)	5 (12.2)	.165
30-49 y	90 (31.0)	76 (30.5)	14 (34.1)	-
50-69 y	120 (41.4)	107 (43)	13 (31.7)	-
≥70 y	64 (22.1)	55 (22.1)	9 (22.0)	-
Sex—No. (%)				
Female	135 (46.6)	110 (44.2)	25 (61.0)	.062
Male	155 (53.4)	139 (55.8)	16 (39.0)	-
Exposure History—No. (%)				
Yes	83 (28.6)	74 (29.7)	9 (22.0)	.308
No	207 (71.4)	175 (70.3)	32 (78.0)	-
Comorbidity—No. (%)	178 (61.4)	158 (63.5)	20 (48.8)	.074
Hypertension	81 (27.9)	68 (27.3)	13 (31.7)	.561
Diabetes mellitus	27 (9.3)	25 (10)	2 (4.9)	.393
Coronary heart disease	18 (6.2) (6.2)	18 (7.2)	0 (0.0)	.087
Drug hypersensitivity (self-reported)	10 (3.4)	10 (4.0)	0 (0.0)	.367
COPD	6 (2.1)	6 (2.4)	0 (0.0)	.600
Urticaria	2 (0.7)	1 (0.4)	1 (2.4)	.263
Asthma	1 (0.3)	1 (0.4)	0 (0.0)	>.999
Others	72 (24.8)	66 (26.5)	6 (14.6)	.103
Surgery history—No. (%)	89 (30.7)	82 (32.9)	7 (17.1)	.041
Tumor surgery	15 (5.2)	15 (6.0)	0 (0.0)	.141
Craniocerebral surgery	6 (2.1)	6 (2.4)	0 (0)	.600
Cardiac intervention	10 (3.4)	10 (4.0)	0 (0)	.367
Others	62 (21.4)	55 (22.1)	7 (17.1)	.468
Smokers—No. (%)	28 (9.7)	25 (10)	3 (7.3)	.778
Past Smokers	18 (6.2)	16 (6.4)	2 (4.9)	>.999
Current Smokers	10 (3.4)	9 (3.6)	1 (2.4)	>.999
Severity—No. (%)				
Severe	121 (41.7)	111 (44.6)	10 (24.4)	.015
Nonsevere	169 (58.3)	138 (55.4)	31 (75.6)	-

Note: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; rRT-PCR, real-time reverse transcription–polymerase chain reaction; COPD, chronic obstructive pulmonary disease; *P* values denote the statistical significance between initially positive and negative result subgroups.

tract is higher than in the upper respiratory tract.[9] Currently, most samples are collected from the upper respiratory tract, such as oropharynx swabs, due to ease of sampling or absence of sputum in the patient, which may lead to false-negative rRT-PCR results. Another important point to emphasize here is that the same errors for initial negative results can occur at the time of decision to discharge for clinically healed patients. As can be seen, the patients involved in the study are all hospitalized cases and we had a chance to further confirm the data with clinical diagnosis and

repeated rRT-PCR tests. Some of the false-positive results can be because of low virus expression in pharynx samples, which can be even higher percentages in nonhospitalized cases. In this context, the isolation of COVID-19 suspected patients should be more vigorous and the decision should not only depend on rRT-PCR positivity during the time of the pandemics.

The findings presented herein suggest that a considerable proportion of COVID-19 patients may have an initial negative rRT-PCR result and that initially positive patients had a higher tendency to progress

to severe cases. Therefore, diagnosis of SARS-CoV-2 infection should not be excluded in patients with an initial negative rRT-PCR result, especially when presented with typical clinical manifestations. In view of these results, we recommend repeated rRT-PCR tests to confirm diagnosis and identify potentially infected individuals.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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